THE EMBRYOLOGY AND SEEDLING DEVELOPMENT OF
AEGICERAS MAJUS GAERTN.

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(Thirty-two Text-figures.)

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Introduction.

For many years vivipary has been recognized as a feature of the mangroves of the world. Treub (1883) was the first to undertake an investigation of the development of the seedlings by tracing the embryology of *Avicennia officinalis* Linn. in all its stages. Later Haberlandt (1896) examined the methods of nutrition of the viviparous embryos and the seedlings of *Bruguiera eriopetala*, *Rhizophora mucronata* and *Aegiceras majus*. His observations regarding *Aegiceras majus* were made on material in which the youngest ovule was already 7 mm. long. The writers, having had access to much younger material, find that the early stages in the development of the seed show several additional points of interest. These, combined with Haberlandt's detailed account of the older embryo, give a complete account of the growth of the seed and seedling of this species. In addition, they afford an interesting comparison with the stages of development in *Avicennia officinalis* as described by Treub.

Aegiceras majus Gaertn. is a low shrub or small tree. It is frequently found associated with *Avicennia officinalis* Linn. on the marshy flats or the sloping banks of the coastal rivers in the Sydney district. This has already been recorded by Collins (1921). Where it is present in any abundance it may form a definite belt behind a zone of *Avicennia*. The size of the plant varies considerably, and there appear to be two fairly well defined growth forms present. Those plants which grow on the tidal flats skirting the coastal harbours, and whose roots are hence swept twice daily by salt water, are as a rule small rigid shrubs rarely exceeding 5 feet in height. The smallest plants are 1–2 feet high and are found in the most exposed situations. More sheltered plants growing along the banks of tidal rivers in brackish water may attain a height of 10–12 feet and are much more slender than the robust harbour forms. The habitat of this species is thus an area submerged by the rising tide under salt or brackish water.

Aegiceras majus is known as the "river mangrove". It extends north into Queensland, and is found also along the coasts of the old world tropics. Although it is frequently not as abundant as *Avicennia officinalis*, it can be distinguished easily from it at a distance by the bright green colour of the foliage.

This genus is the only member of the Myrsinaceae which is present in the mangrove flora. Its characteristic flowers have been so fully described by systematists (Engler and Prantl, 1891) that any further description is unnecessary. The flowering period begins in early winter (May-June) and extends over several months. The phases immediately following fertilization proceed slowly, so that it is not till early December that endosperm formation is begun in the embryo sac. From this time development proceeds more rapidly, so that in late January the young embryo is sufficiently developed to emerge into the cavity of the ovary, and by March or April is about 3 cm. in length and ready to be shed from the tree. Owing to the fact that the period of fruit development extends over about a year, flower buds may be initiated before all the fruits of the previous season have been shed.

The smaller or "shrub" forms are found to produce larger fruits than the "tree" forms. The fruits are about ½-1 cm. longer, and proportionately thicker than those produced on the larger trees.

The Gynoecium.

The gynoecium is a flask-shaped structure, the style merging imperceptibly into the ovary. The latter has a conical loculus into which projects a free basal placenta (Text-fig. 1). Many of the cells in the wall of the ovary and in the style and placenta contain a substance, in the form of finely divided droplets, which, after fixation in formalin acetic alcohol, becomes yellow. This substance was tested by means of the standard microchemical methods (Haas and Hill, 1928, p. 79), and was found to give reactions for both proteinaceous and fatty material.

The stylar region of the gynoecium is studded with schizogenous glands (G, in Text-fig. 1). These may also be present occasionally in the ovary wall. Another feature of the style is the occurrence of young sclereids in whose walls are numerous simple pits. Here and there in the walls of the loculus, and particularly on the lower part of the expanded apex of the placenta, are glandular hairs. These hairs each have a globular head composed of a number of densely protoplasmic cells, borne on a stalk of 2-4 cells (Text-figs. 2a, 2b). Thus they differ appreciably from those which have been figured by Solereder from the foliage of the same species, and described by him (1908, pp. 610–611) "as especially peculiar in having their lateral walls fused with the wall of the depression and in the arrangement of the ray cells in surface view, which remind one of certain corals (*Fungia discus*)".

The placenta, arising at the base of the ovary, has, at the time of fertilization of the ovules, a short stalk which projects into an expanded apex. This in longitudinal section has the appearance of an arrow-head (Text-fig. 1). A trans-

Text-fig. 2.—A, glandular hair from the wall of the ovary or placenta. B, surface view of the head of a glandular hair. × 250.

Text-fig. 3.—A transverse section of the expanded part of the placenta. E, lower part of the expanded apex of the placenta; O, ovule; T, stalk of placenta; V, vascular strand. × 49.

Text-fig. 4.—A tangential section of a young ovule. P, peripheral layer of the integument; I, integument; N, nucellus; M, micropyle. × 250.

Text-fig. 5.—A radial section of a young ovule at the time of fertilization. I, integument; P, peripheral layer of the integument; N, nucellus; E, embryo-sac; F, funicle; M, micropyle. × 250.

Text-fig. 6.—A radial section of an ovule enlarging after fertilization. I, integument; P, peripheral layer of integument; N, nucellus in process of being resorbed; E, embryo-sac; F, funicle; M, micropyle. × 250.
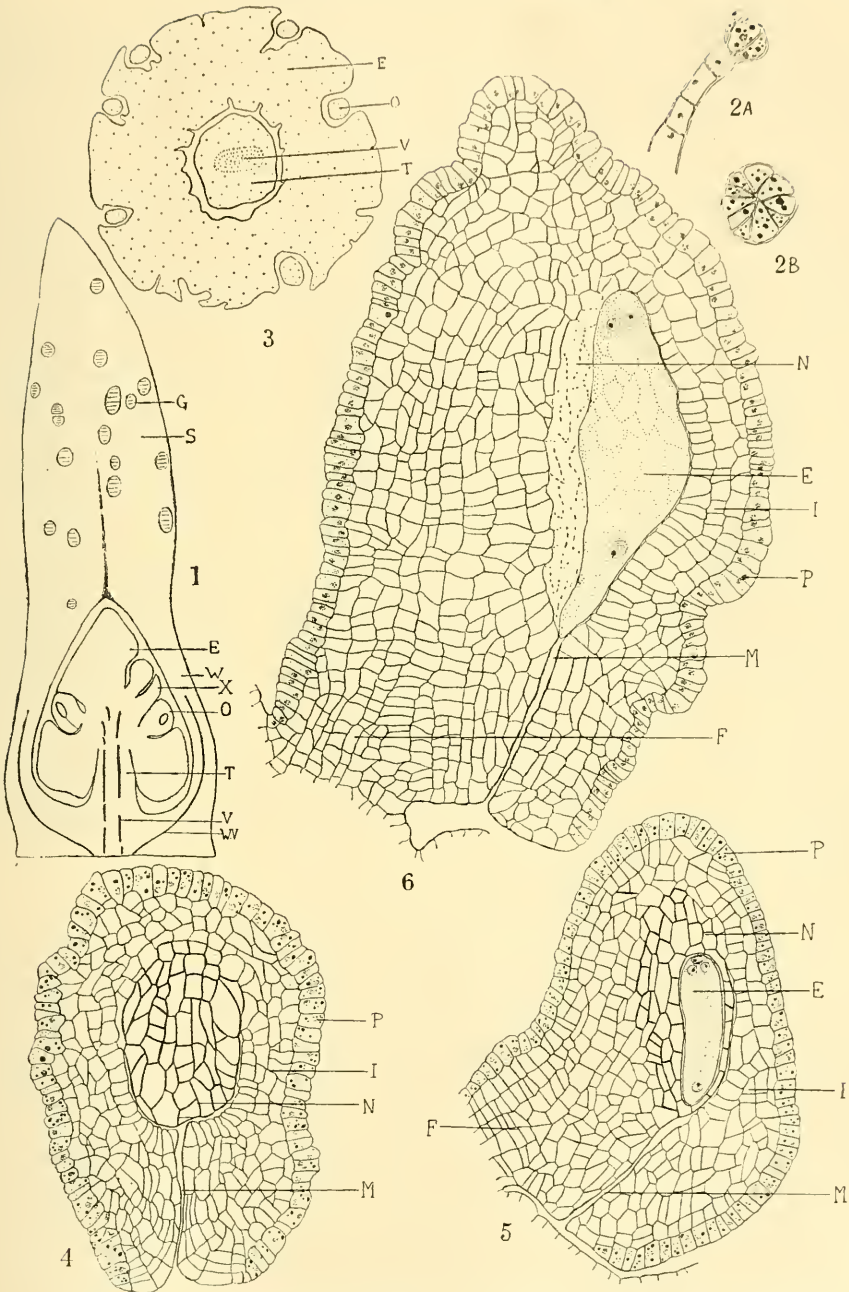
Text-fig. 1.—A longitudinal section of the ovary and part of the style.
G, schizogenous gland; S, style; W, ovary wall; E, expanded part of the
placenta; V, vascular strand of the placenta; WV, vascular strand of the
ovary wall; X, projection of placenta tissue between ovules; O, ovule; T, stalk
of placenta. × 19.

verse section passing through the upper region of the stalk (Text-fig. 3) shows the
expanded apex as an apparently free tissue around the stalk. In this expanded
apical region numerous ovules are embedded (Text-figs. 1, 3) so that there are
projections of the placenta between them.

The Ovule at Fertilization.

Each ovule is anatropous and has a single massive integument (Text-fig. 5).
Through this integument extends a long narrow micropyle which, as the funicle
is very short, faces the placenta. The outermost layer of the integument is clearly
differentiated from the inner layers, as the cytoplasm of the cells is denser, and
globular food-bodies with the same staining properties as those mentioned pre-
viously in the placenta are clearly visible. The cells of the integument below
the embryo-sac and those in the funicular region are arranged in fairly regular
rows (Text-figs. 4, 5). As will be seen later, these cells are potentially
meristematic.

There is no trace of vascular tissue in the funicle, so the ovule is without
a conducting strand. The only vascular supply is found in the placenta, and
here the centrally placed tracheids can be traced to a position almost level
with the uppermost ovule (Text-fig. 1).

The nucellus is composed of cells which are larger and thicker-walled than
those of the integument which invests it. Also the degree of fusion of the
integument and the nucellus varies slightly. Text-figure 4 shows the nucellus
free on both sides to about the median region of the embryo-sac, but frequently
the nucellus on the outer side of the embryo sac is free to a point level with
the base of the sac (Text-fig. 5).

The embryo-sac is elliptical in section and is excentrically placed in the
nucellus, so that, on the side of the ovule remote from the funicle, there are
only one or two rows of cells between it and the integument. The embryo sac
previous to fertilization shows the eight nuclei typical of most dicotyledonous
embryo-sacs.

Post-fertilization Stages.

Fertilization may occur in a number of ovules simultaneously in any one
ovary. After fertilization, the ovule enlarges and the embryo sac increases in size
at the expense of the nucellus. The nucellus on the side of the embryo-sac
remote from the funicle, being thinner than that on the funicular side, becomes
resorbed first. At one stage of its development, therefore, the embryo-sac appears
to have nucellar tissue on the inner side only (Text-fig. 6). All traces of the
nucellus are, however, soon lost and at this stage the embryo-sac is bounded
only by the integument. The cells of the integument seem to offer a greater
resistance to the growth of the embryo-sac than do the cells of the nucellus, as
they are not affected by the resorbing action of the sac. This resistance causes
the further extension of the embryo-sac, therefore, to be directed into the micropyle
(Text-fig. 7). At the same time cell-division begins in the integument in the
micropylar region, and proceeds with increasing rapidity. The embryo-sac con-
tinues to invade the micropyle, and so pushes down into this meristematic region
(Text-figs. 7, 8). Successive divisions in this tissue result in the rapid elongation
of the micropylar part of the integument, but the bulk of the tissue so formed is
resorbed by the advancing embryo-sac. This resorption proceeds transversely as

well as longitudinally in the micropylar region of the ovule, so that the integument here, i.e., below the original embryo-sac, is gradually reduced to a single layer, except at the outer end of the micropyle. In this region it still remains massive and cell-divisions take place so that the tissue keeps pace with enlarging sac. Meristematic activity extends gradually from the region of the micropyle to the adjacent cells of the funicle, so an increase in size takes place here also. Remnants of the inner part of the integument persist for some time between the embryo-sac and the intact outer layers of the integument. At this stage in the development, the greater part of the ovule is occupied by the enlarged embryo-sac (Text-figs. 9, 10), whilst towards the apex of the ovule the outline of the original embryo-sac is retained by the integumental cells. At quite an early stage the cells of the
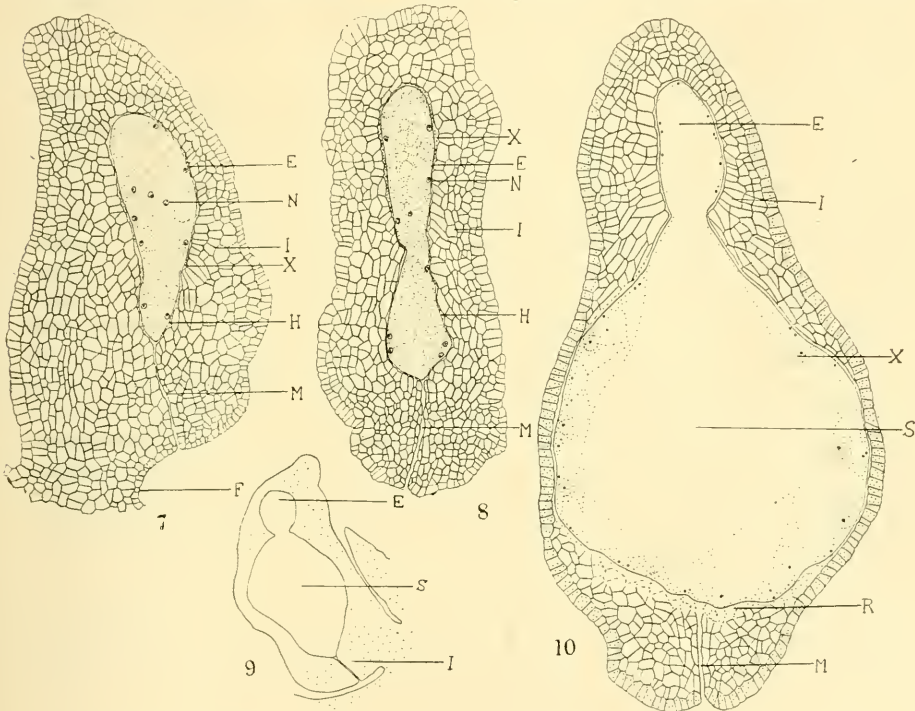
Text-fig. 7.—A radial section of a fertilized ovule showing the embryo-sac commencing to grow into the micropyle. I, integument; X, resorbed nucellus; E, embryo-sac; N, endosperm nuclei; H, haustorial part of the sac; M, micropyle; F, funicle. × 124.

Text-fig. 8.—A tangential section of a fertilized ovule at a slightly older stage than that shown in Text-fig. 7. I, integument; X, resorbed nucellus; E, embryo-sac; N, endosperm nuclei; H, haustorial part of the sac; M, micropyle. × 124.

Text-fig. 9.—A radial section of a fertilized ovule showing the embryo-sac much enlarged in the micropylar region. E, original position of embryo-sac; S, extension of the sac into the micropyle; I, integument. × 41.

Text-fig. 10.—A tangential section of a fertilized ovule at a stage similar to that shown in Text-fig. 9. I, integument; E, original position of embryo-sac; S, extension of the sac into the micropyle; X, endosperm nuclei; R, resorbed integumental tissue; M, micropyle. × 114.

funicle and the adjacent massive micropylar integument become filled with the characteristic proteinaceous food material.

During the enlargement of the embryo-sac the endosperm nucleus has undergone repeated divisions, giving rise to numerous small nuclei. The nuclei at first lie scattered throughout the embryo-sac (Text-figs. 7, 8), and can be clearly distinguished from the larger zygote nucleus, which is at the micropylar end of the sac. However, as the embryo-sac enlarges, the cytoplasm at the centre becomes very attenuated and the nuclei move to the periphery, at the same time increasing in size. This enlargement of the nuclei can be seen if Text-figures 7 and 8 ($\times$124) are compared with Text-figure 10, which is drawn on a slightly smaller scale ($\times$114) and yet shows obviously larger nuclei. At this stage the zygote nucleus can no longer be identified, but it apparently retains its position at the micropylar end of the advancing embryo-sac as the initial embryonal tissue makes its appearance in that position.

Several fertilized ovules may reach this stage of development, but one ovule, usually one situated towards the apex of the placenta, soon becomes dominant.

In the now single layer of the integument, divisions at right angles to the surface of the ovule take place so rapidly that this tissue falls into folds around the embryo-sac (Text-fig. 11A). At the same time cell wall formation begins at the periphery of the sac and advances inward till an endospermic tissue is formed. This tissue is composed of small cells at the periphery merging into larger central cells (Text-fig. 11B). The position of the original embryo-sac is still visible (Text-fig. 11A), and can be traced through the various stages of development till the time when the folds in the integument are straightened out by the growing embryo and endosperm. Meanwhile meristematic divisions continue in the funicle (F, in Text-fig. 11A) and in the adjacent part of the integument, and result in the widening of that area. The cells composing it are filled with reserve food material similar to that described in the integument of the young ovule. This material stains strongly with most reagents, e.g., safranin and haematoxylin, and is present in such quantities that after staining no detail of cell structure can be made out unless the sections are so destained that all other parts become almost colourless.

The embryo itself remains indistinguishable previous to the formation of endosperm tissue in the embryo-sac. It can be observed at the micropylar end of the embryo-sac, when endosperm cell formation is completed, and is at first quite close to the periphery of the sac (Text-fig. 12).

The small endosperm cells comprising the peripheral zone in the embryo-sac are more densely protoplasmic than the inner cells, and become meristematic

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Text-fig. 13.—A young embryo embedded in the endosperm. X, inner primary endosperm; R, endosperm in process of being resorbed; E, embryo; S, suspensor; M, meristematic region of the endosperm; C, crushed inner layer of the integument; I, integument. $\times$ 280.

Text-fig. 14.—A. A view of the expanded portion of the placenta on which can be seen the aborted ovules (A) and a fertilized ovule at the stage shown in Text-fig. 12. O, position of original embryo sac; S, enlarged portion of the sac; P1, furrowed basal part of the expanded apex of the placenta; P2, tip of the placenta. $\times$ 10 approx.

B. A view of the placenta and developing seed taken at right angles to that shown in Text-fig. 14A. O, position of original embryo sac; S, enlarged part of the sac; A, aborted ovules; P1, furrowed basal part of the expanded apex of the placenta. $\times$ 10 approx.
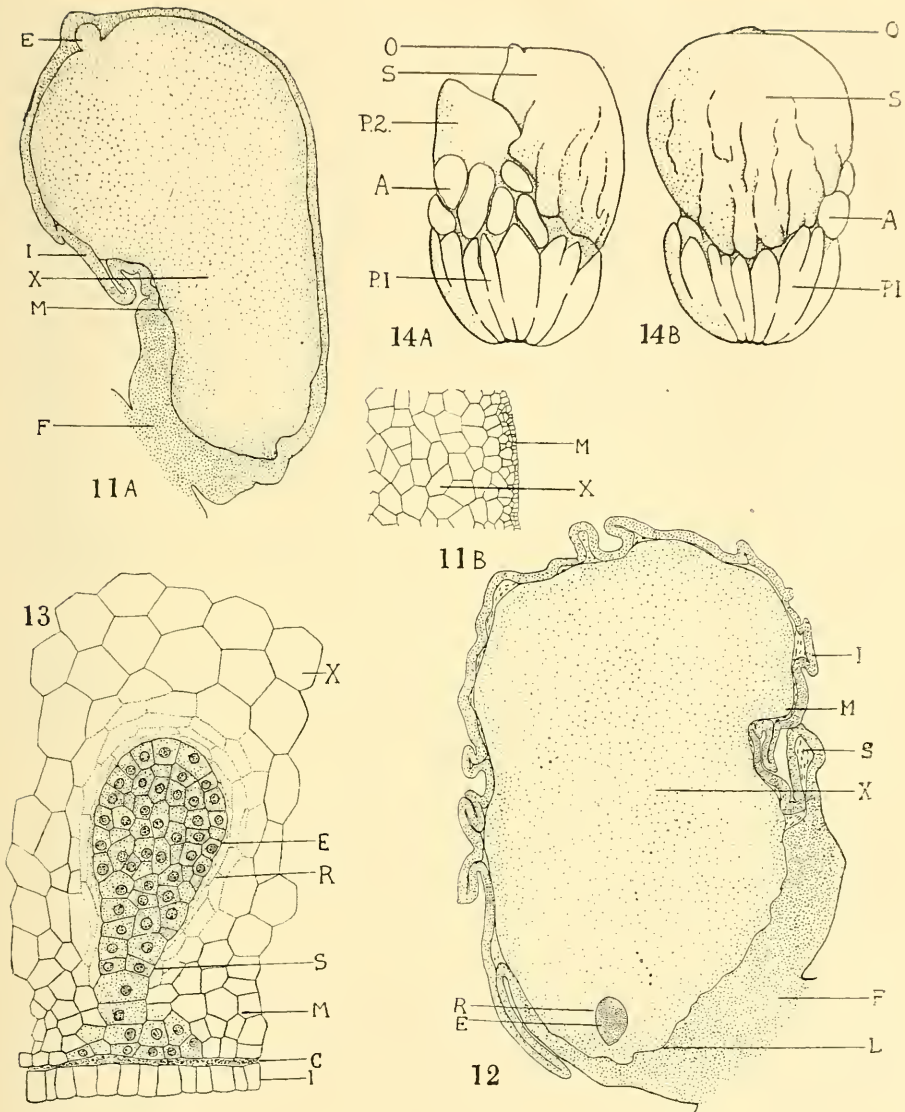
Text-fig. 11.—A. A radial section of a fertilized ovule in which endosperm tissue has been formed. I, outer layer of the integument; E, original position of the embryo-sac; X, inner endosperm; M, outer endosperm; F, funicle. × 30.

B. Part of Text-fig. 11A shown in full cell detail. M, outer endosperm; X, inner endosperm. × 30.

Text-fig. 12.—A radial section of a developing seed showing the enlarging embryo and the differentiation of the endosperm into an outer and an inner region. I, outer layer of the integument; S, fragments of the inner part of the integument; M, meristematic region of the endosperm; X, inner primary endosperm; R, resorbed zone of the endosperm; E, embryo; F, funicle; L, endospermic lobes. × 30.

soon after their formation. By their activity new cells are formed, at first mainly in radial rows at right angles to the outer wall of the embryo-sac, but later cells are cut off in all directions in this peripheral zone. The new cells remain small, and thus a very distinct zone is formed around the margin of the endosperm (Text-fig. 13). Meristematic activity in this peripheral zone of the endosperm continues rapidly, with the result that the endosperm tissue increases in size. The result of this increase in size is particularly striking in the lower part of the ovule. Here the endosperm is in contact with the massive meristematic and food-filled tissue of the funicle and with the basal part of the integument in the micropylar region. When the outer endosperm becomes meristematic it also assumes a haustorial function. By continued cell division it forces its way into the massive tissue in a series of lobes (L, in Text-figs. 12, 15). These lobes in their mature condition have been fully described by Haberlandt. They increase in length and width, boring steadily into the adjacent tissues; sometimes branching into finger-like processes; sometimes twisted so that in section there appear to be isolated islands of meristematic haustorial endosperm in the tissue of the integument or funicle. It is evident that these endospermic folds resorb the integumental cells with which they come in contact, but there is very little evidence of crushing or alteration of tissue in advance of the folds. They are in very close contact with the cells of the funicular and integumental tissue they invade. In contrast with this, traces of crushed cells are often seen between the endosperm and integument on the flanks and top of the endosperm.

In spite of the haustorial action of the endospermic folds, the thickness of the invaded tissue of the integument and funicle is maintained and even increased by continued cell division in all planes. Cell division in the rest of the peripheral zone of the endosperm, i.e., in the upper part of the seed, away from the micropyle and funicle, is not so marked as in the lower part. It results in a general increase in size of the developing seed.

Meristematic activity in the endosperm is practically limited to the outer 6–7 rows of cells, so that a secondary tissue is formed behind the meristematic region by its continued advance. The cells of this tissue remain small and thin-walled and constitute what may be referred to as the secondary endosperm, in contrast with the large-celled primary endosperm filling the centre of the embryo-sac.

At the same time the remaining single row of cells constituting the major part of the integument continues to divide rapidly in a plane at right angles to the surface of the ovule, so that it becomes more and more wrinkled over the surface of the endosperm, in spite of the continued growth of that tissue (Text-fig. 12).

The embryo rapidly increases in size, and by the time meristematic activity is established in the peripheral endosperm, the divisions of the fertilized egg have resulted in the formation of an embryo such as is shown in Text-figure 13.

A study of the seed at this stage of development (i.e., at the stage indicated in Text-fig. 12) shows that the much enlarged embryo-sac is not spherical, but is rather spheroidal in shape. Looking at the seed in radial view, it appears fairly narrow, lying obliquely on the placenta, with the original embryo-sac visible at the apex (Text-fig. 14A), while from another aspect at right angles to the first (Text-fig. 14B), it appears much wider and the heavy folding of the integument is more clearly shown. The growth of the seed is already pushing the apex of the
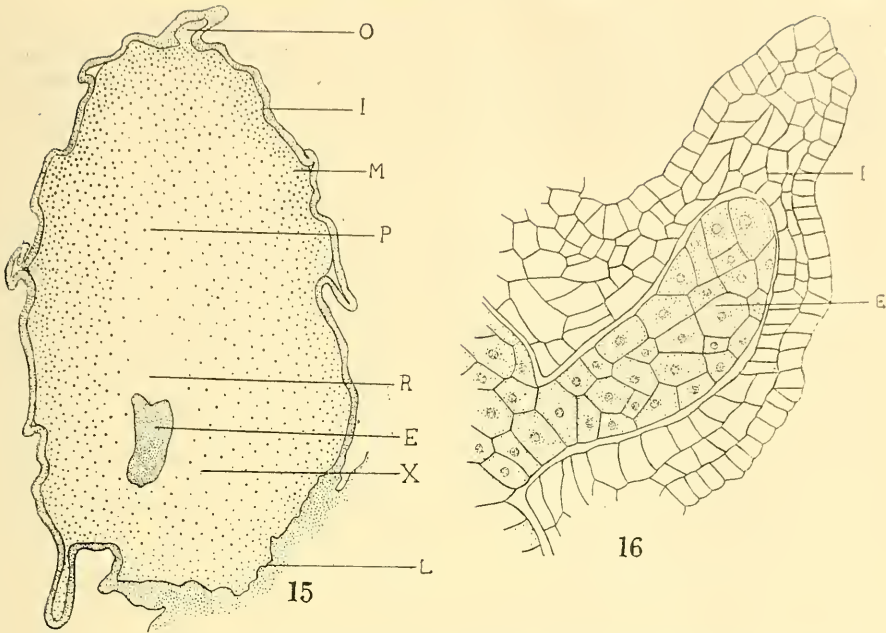
Text-fig. 15.—A radial section of an older seed than that shown in Text-fig. 12. O, position of the original embryo-sac; I, integument; M, meristematic region of the endosperm; E, embryo; X, endosperm which has been attacked by enzymes; L, endospermic lobes; R, primary endosperm in process of being resorbed; P, unaltered primary endosperm. × 30.

Text-fig. 16.—The original embryo-sac of Text-fig. 15 shown in detail. I, integument; E, endosperm. × 262.

arrow-shaped head of the placenta to one side of the loculus of the ovary. The lobing and furrowing characteristic of the lower part of the apex of the placenta is also clearly shown in Text-figures 14A and 14B. In the median region of the expanded apex of the placenta (Text-fig. 14A) many aborted ovules can be seen.

The Growth of the Embryo.

The embryo increases in size at the expense of the neighbouring endosperm cells. These endosperm cells are first altered by the action of enzymes secreted by the embryo, since, although they are quite undeformed, cells at a considerable distance from the embryo show evidence of alteration by their reaction to stains. The altered endosperm cells are then crushed by the growth of the embryo and their contents are resorbed. Around the growing embryo, therefore, there are a number of zones. Firstly, adjacent to the embryo, are the structureless remains of the cells whose contents are being resorbed, cell walls are disintegrating and nuclei are no longer visible (R, in Text-fig. 17); further out is a zone of cells whose contents appear homogeneous, due to enzyme action, and which are slightly deformed by the pressure of the growing embryo (U, in Text-fig. 17); further out still, is the zone of undeformed cells which are in the process of alteration by enzyme action (X, in Text-fig. 17), and this grades into the unaltered endosperm. The altered zones are readily distinguished in stained sections, since they stain

practically uniformly with dyes such as anilin blue and Delafield's haematoxylin. At the periphery of the endosperm the meristematic zone of densely protoplasmic cells is evident (M, in Text-fig. 17), while between this zone and the integument, crushed fragments of the cells which composed the inner layers of the integument can sometimes be made out.
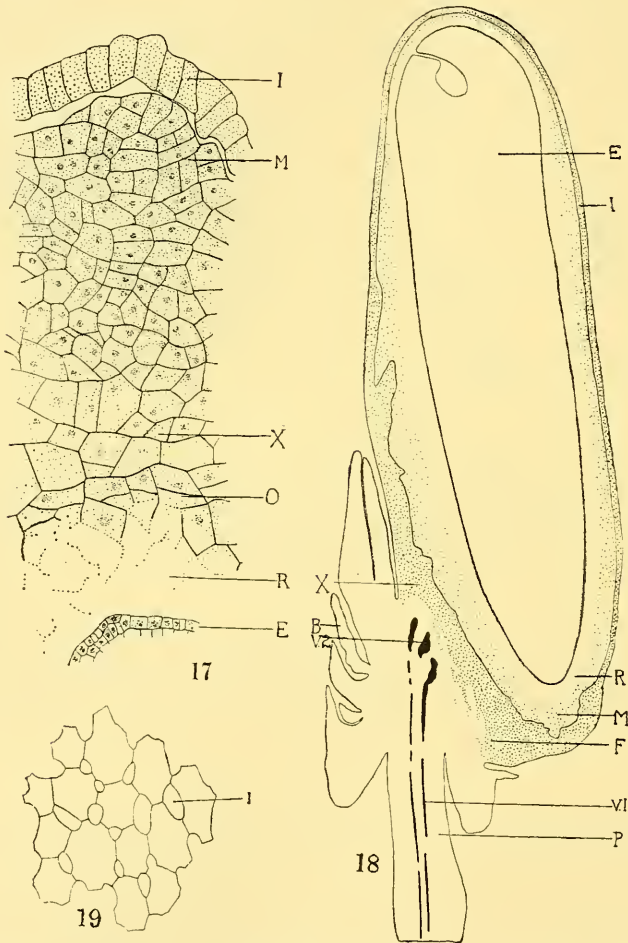
Text-fig. 17.—Part of the developing seed shown in Text-fig. 15 shown in full cell detail. I, integument; M, meristematic region of the endosperm; X, endosperm which has been attacked by enzymes; O, slightly deformed endosperm; R, resorbed endosperm; E, embryo. × 250.

Text-fig. 18.—A radial section of a seed and the placenta showing a much enlarged embryo which extends the full length of the seed. I, integument; E, embryo; R, inner endosperm in the process of resorption; M, outer meristematic and haustorial endosperm; F, funicle; B, aborted ovules; P, placenta; V1, conducting tissue; V2, vascular tissue just below the funicle. × 19.

Text-fig. 19.—A small portion of a tangential section of the stalk of the placenta showing intercellular spaces (I). × 267.

The young embryo grows rapidly, and by the time it is 0·4 mm. in length it begins to show signs of the development of cotyledons (Text-fig. 15). Text-figure 15 also shows the original position of the embryo-sac at O, at the apex of the ovule. This part is shown in detail in Text-figure 16. The integument (I) is thicker here than on the flanks of the ovule, and the position of the original embryo-sac is filled with a small-celled prolongation of the endospermic tissue (E).

As the embryo increases in size the primary endosperm becomes wholly resorbed and the secondary endosperm is next attacked. The subsequent growth of the embryo is very rapid, and to accommodate it and provide the necessary food material, the growth of the haustorial and meristematic endosperm is also very rapid, the haustorial lobes becoming very much increased in size and complexity. Continued divisions of the cells of the massive region of the funicle and integument enable this tissue to maintain its dimensions and so still provide the food supply for the haustorial and primary endosperm and the embryo. Divisions in the meristematic endosperm along the flanks and over the apex of the ovule enable it to keep pace with the rapidly enlarging embryo, but during this process it becomes very thin (Text-figs. 18, 21). This enlargement of the embryo and endosperm causes the folds in the integument to be stretched out (Text-fig. 18), so that this layer soon fits very tightly over the surface of the endosperm.

The haustorial folds of the endosperm are now very numerous. In most cases there are a relatively few major haustoria such as are shown in Text-figure 21, and from these, numerous small subsidiary haustoria develop into the integument and funicle. At the stage shown in Text-figure 21, these haustoria have rather blunt apices consisting of a number of small cells. Occasionally one finds narrow haustoria one or two cells wide forcing their way into the funicle. The major folds seem to be developed chiefly vertically in a plane tangential to the placenta, since they are best seen in tangential longitudinal sections. Transverse sections taken just below the vascular strand of the placenta (about X, in Text-fig. 18) show the subsidiary folds excellently, and the position of a major fold may be indicated by a wide shallow extension of the endosperm. Radial sections frequently miss the major folds altogether, and show only the smaller ones (Text-fig. 18). When the embryo is nearing maturity and has emerged into the cavity of the ovary, the cells at the ends of the haustoria tend to grow out separately for short distances. This development is specially well seen in transverse sections taken just above the vascular strand of the placenta, and has been very adequately described by Haberlandt. In transverse sections which include the vascular strand of the placenta, individual haustorial lobes are less numerous and less deeply penetrating than in sections taken higher up. The endosperm tissue encroaches on this part of the funicle in an almost unbroken front. In contrast to this Haberlandt found that in the ovules examined by him the haustorial lobes were more pronounced here than elsewhere.

The contact between the cells of the endospermic folds and the cells of the massive tissue they invade is, as was described before, very close, so that without a knowledge of the previous history of the two tissues, one would have difficulty in distinguishing them as of separate origin. In Text-figure 20 a small section of this region is shown in detail; at F, are the thin-walled dividing cells of the funicle, which are filled with darkly staining reserve food and are arranged in more or less definite rows; at M, are the cells of the haustorial endosperm, also
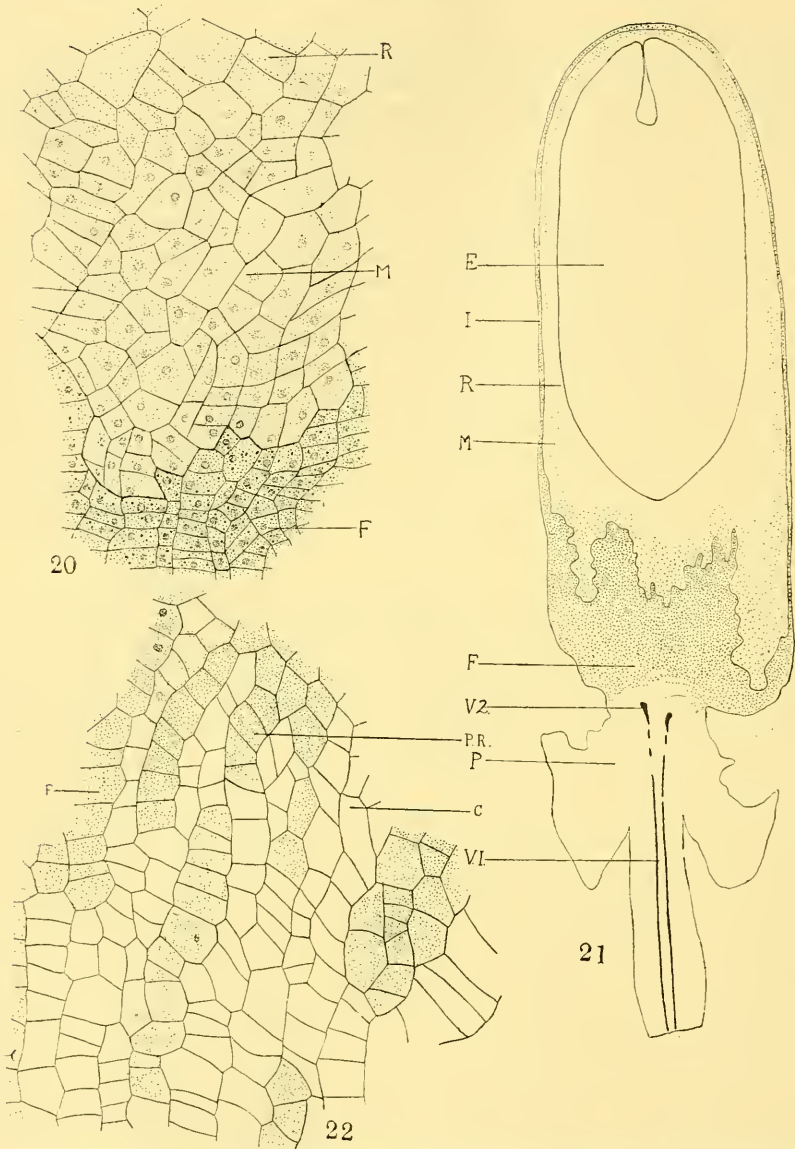
Text-fig. 20.—Part of a radial section of a seed showing the full cell detail of the meristematic region of the funicle (F). M, haustorial endosperm; R, inner endosperm. × 250.

Text-fig. 21.—A longitudinal section passing through the placenta and tangentially through the upper part of the embryo. The embryo is slightly older than that shown in Text-fig. 18. E, embryo; I, integument; R, inner endosperm undergoing resorption; M, haustorial endosperm; F, funicle; V1, vascular tissue of the placenta; V2, vascular tissue just below the funicle; P, placenta. × 19.

Text-fig. 22.—A detailed study of the part of the placenta adjoining the funicle, showing rows of cells filled with proteinaceous material. F, funicular cells; PR, cells of the placenta filled with proteinaceous material; C, clear cells of the placenta. × 250.

in fairly regular rows, but distinct from the rows of the funicular cells they invade. These cells are thin walled and densely protoplasmic, but stain less darkly than the cells of the funicle, since they have no reserve food. Behind this actively meristematic zone is the region where divisions have ceased and upon which the growing embryo is encroaching. Haberlandt (1895) has given a detailed account of the embryo at about this stage of development, but since the embryos examined by the present writers differ slightly from those described by him, Haberlandt's observations will be discussed later, and a complete description is given here of the facts as observed by us.

During the period of enlargement of the embryo, the stalk of the placenta increases in length (cf. Text-figs. 18 and 21). This process seems to be simply one of cell expansion, the individual cells becoming larger and broader, and air spaces appearing between them (Text-fig. 19).

To accommodate the increase in size of the funicle, meristematic divisions take place in the adjacent part of the placenta. New elements are added to the vascular strand which extends towards the funicle but never into it, or into the integument. It is obvious, therefore, that food material for the growing embryo must first pass across the small cells of the funicle and thence be absorbed by the haustorial endosperm. The cells of the funicle are small, fairly thin walled and stain darkly because of the presence of food material within them. The cells of the placenta are similar in size and shape, but food material is stored only in a few scattered cells, so that the tissue as a whole does not stain darkly. The two tissues are, therefore, fairly sharply differentiated from one another. Where they merge it is often found that rows of cells filled with proteinaceous food material and rows of clear cells alternate over a short area (Text-fig. 22).

By the time the embryo has reached a length of 5 mm. (Text-fig. 18) the cotyledons are fully differentiated. They are short and fleshy, and in longitudinal section appear triangular. Occasionally they are slightly twisted around one another. The meristem of the radicle is also distinguishable at this stage, but remains dormant and undifferentiated until the embryo begins to germinate. The embryo by this time has become a deep green, due to the presence of chlorophyll in the cells of the cotyledons and the cortex of the hypocotyl. In older specimens multicellular hairs, as described by Haberlandt (1895), are present around the young radicle. The plumule remains as an undifferentiated meristem between the cotyledons until the young plant becomes established in the soil.

Up till the stage shown in Text-figures 18 and 21, increase in size is due to cell divisions throughout the whole embryo, but now growth becomes practically confined to the lower part of the hypocotyl. The radicle, therefore, becomes gradually pushed down to a position below the apex of the placenta (Text-fig. 23). The integument and endosperm keep pace with this extension in length by continued cell division. But eventually the elongation of the hypocotyl causes the radicle to break through the integument at the lower end of the ovule so that it emerges into the cavity of the ovary. At the same time the stalk of the placenta continues to elongate, so that the integument and the endosperm remain as a close fitting cap over the cotyledons and upper part of the hypocotyl. This condition is shown in Text-figures 24 and 25 (see also Haberlandt, 1895, Text-fig. 8). The embryo by this time completely fills the ovary, and the placenta, endosperm and integument are crushed very closely against it. As the embryo approaches its mature size (Text-fig. 25) it becomes curved, with the placenta along the concave side. This bending appears to be due to the fact that the
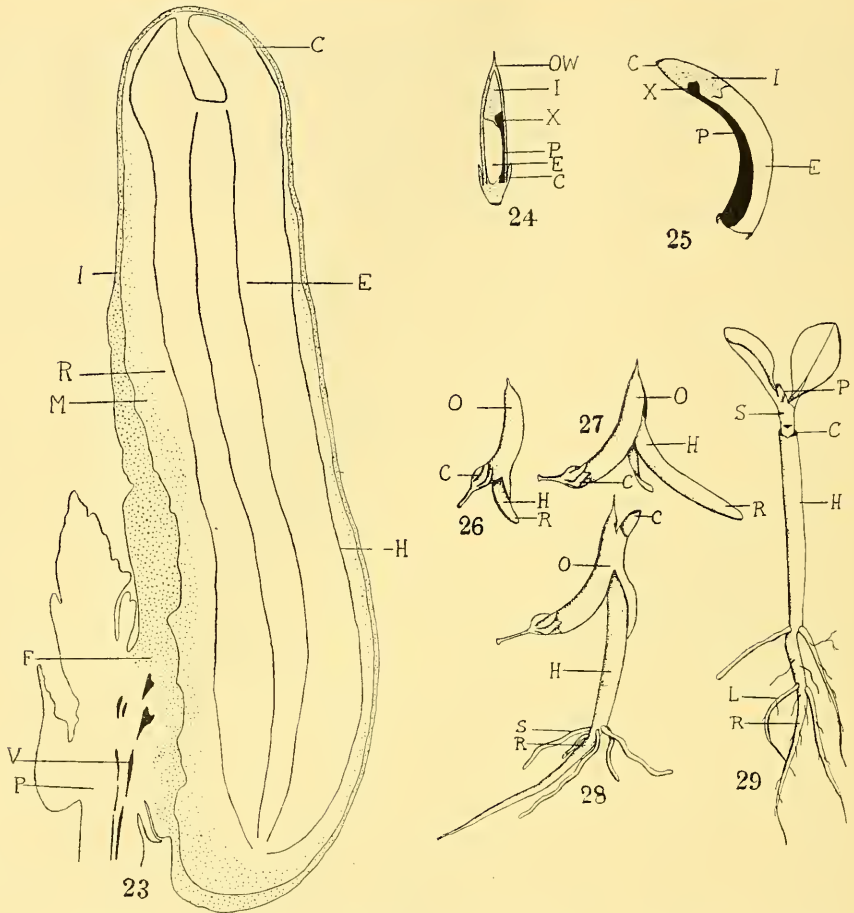
Text-fig. 23.—A radial section of a seed older than that shown in Text-fig. 18. The extension of the hypocotyl has carried the base of the embryo below the level of the apex of the placenta. E, embryo; C, cotyledons; I, integument; R, inner resorbed endosperm; M, haustorial endosperm; H, hypocotyl; F, funicle; P, placenta; V, vascular tissue. × 19.

Text-fig. 24.—A diagram showing the young embryo within the ovary. Part of the ovary wall is removed. OW, ovary wall; I, integument; X, upper part of the placenta and aborted ovules; P, placenta; E, embryo; C, persistent calyx. × 0·7.

Text-fig. 25.—An embryo removed from the fruit. C, tip of the cotyledons; E, embryo; I, integument; X, upper part of the placenta and aborted ovules; P, placenta. × 0·95.

Text-fig. 26.—An embryo emerging from the fruit. R, radicle; H, hypocotyl; C, persistent calyx; O, ovary wall. × 0·6.

Text-fig. 27.—A later stage in the emergence of the embryo from the ovary wall. R, radicle; H, hypocotyl; O, ovary wall; C, persistent calyx. × 0·76.

Text-fig. 28.—An older embryo which has developed secondary roots (S). The cotyledons are emerging from the ovary wall (O). R, radicle; C, cotyledons; H, hypocotyl. × 0·76.

Text-fig. 29.—A well established seedling. P, shoot; S, leaf scar; C, cotyledons; H, hypocotyl; R, main root; L, lateral roots. × 0·48.

stalk of the placenta is unable to extend as rapidly as does the hypocotyl, and consequently causes the embryo to arch away from it.

The embryo has by this stage become packed with starch, which is stored as compound grains throughout the pith and cortex of the hypocotyl. It also contains a considerable amount of proteinaceous reserve food material similar to that described in the funicle of the young ovule. A section taken through the funicle and endosperm at this stage shows that the endosperm is practically all resorbed, and that the walls of the invading haustoria become thickened as described by Haberlandt (1895), and the apices of the haustorial lobes tend to branch into the funicle in finger-like processes one cell wide. The thickening of the walls causes the cells to draw away at the corners, leaving air spaces, till finally they become almost separated from each other and from the funicular cells. When the embryo is mature, the endosperm and integument are almost completely dried up, and remain fitting like a cap over the cotyledonary end of the embryo. In the specimens examined by us the funicle never became wholly depleted of its characteristic proteinaceous reserve food.

An account of the emergence and establishment of the seedling has already been given by Collins (1921) in connection with her work on the mangrove vegetation of the Sydney district (N.S.W.), but for the sake of completeness a brief description of the stages in the development of the embryo up to the time of its establishment in the soil will be included here.

Rapid growth continues in the hypocotyl until the embryo is nearly 3 cm. in length. At about this time the fruit falls from the parent plant, but no great check occurs in the process of elongation of the embryo. It soon bursts through the wall of the ovary (Text-figs. 26, 27) and the radicle end of the embryo emerges. If it should have fallen in a suitable place the radicle quickly grows down into the mud and secondary roots are formed which securely anchor the young plant (Text-fig. 28). The cotyledons then emerge from the ovary wall, casting off the integument, and the plumule grows up between them and expands (Text-fig. 29). The reserve food in the hypocotyl begins to disappear as the young plant increases in size, but a considerable amount is still present at the stage shown in Text-figure 29.

"Appendages."

Curious structures which occupy the position of ovules on the placenta and at first resemble them in certain respects are occasionally met with in sections of ovaries. In a young ovary they present an appearance such as is shown in Text-figure 30. Each has a short funicle-like stalk (F) and is somewhat elongated downwards. The tissue composing it, however, is quite homogeneous, there being no indication of integument or embryo-sac, so that the structure cannot be regarded as an ovule, unless possibly as an abnormal development of nucellar tissue. The outermost layer of cells contains proteinaceous material and stains deeply, resembling the peripheral layer of the integument of normal ovules.

As the ovary increases in size and the fertilized ovules develop, these "appendages" elongate rapidly downwards along the furrows of the expanded part of the placenta. As they grow they become slightly twisted, so that it is impossible to obtain a complete view of a fully developed "appendage" in any one section. That shown in Text-figure 31 is by no means a large specimen. The central cells of the elongated part of this structure become long and narrow, and finally develop close spiral thickenings on their walls, so that a full grown

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"appendage" appeared to have a well developed vascular strand down the centre. These spiral elements reach almost to the tip of the structure, as shown in Text-figure 32, but they extend back through the "appendage" only as far as will bring them into close proximity with a schizogenous gland, which occurs in a position approximately analogous to that of the embryo-sac in the normal ovule. The "vascular tissue", therefore, does not pass through the funicle-like stalk and so has no connection with the conducting elements of the placenta.
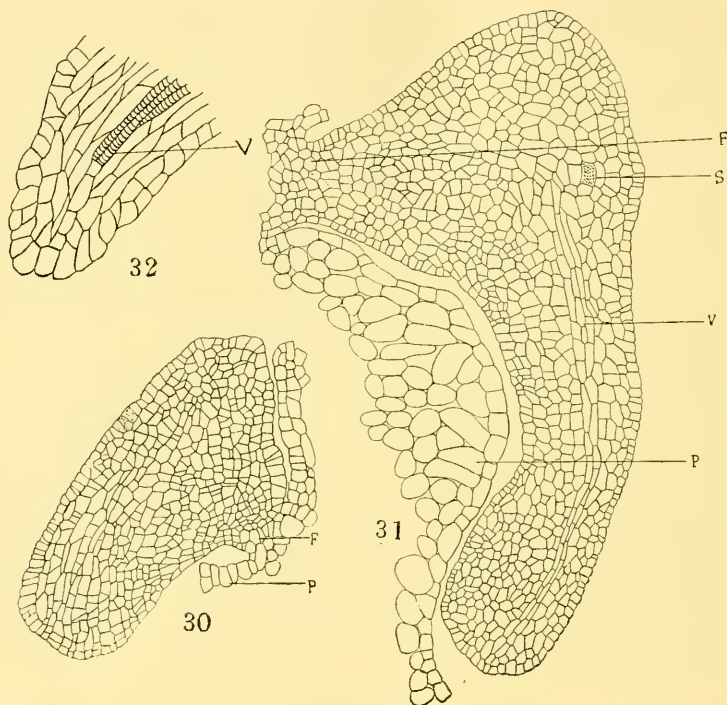
Text-fig. 30.—A young "appendage". F, funicle-like stalk; P, placenta. × 148.

Text-fig. 31.—A later stage in the development of the structure shown in Text-fig. 30. F, funicle-like stalk; S, schizogenous gland; V, elongated cells which later develop spiral thickenings; P, placenta. × 148.

Text-fig. 32.—The tip of an "appendage" older than that shown in Text-fig. 31. V, cells with spiral thickenings. × 250.

The presence of one or more schizogenous glands (S, in Text-fig. 31), similar to those occurring in the ovary wall, is remarkable since no other glands develop at any time in the placenta or normal ovules.

Discussion.

1. *Comparison with Haberlandt's description.*

In his description of the embryology of *Aegiceras majus*, Haberlandt (1895) states that the mature fruit attained a total length of 7 cm., whereas in the Sydney district the mature fruits do not as a rule exceed 3·5 cm. in length, and the "river" forms have even shorter, more slender fruits. As Sydney is almost

the extreme southern limit of the distribution of *Aegiceras*, it is probable that the plants here are much less robust than those growing in the tropics, and that the fruits are correspondingly smaller. This difference in size is probably responsible for some of the discrepancies between Haberlandt's description and that of the present writers.

Haberlandt states that in ovules whose embryos were 7 mm. long the "Schleimendosperm", i.e., the inner endosperm which is in the process of being resorbed, attains a thickness of 0·2 to 0·3 mm. opposite the placenta. In ovules of similar size examined by the writers, the width of this endosperm was never more than 0·1 mm. Since, however, the mature fruits of the form examined by Haberlandt were about twice the size of those examined during the present investigation, Haberlandt's embryo 0·7 mm. in length was probably at an earlier developmental stage than an embryo of similar size collected near Sydney. Also, it might be reasonable to expect a more massive endosperm in the tropical form than in the southern form.

Haberlandt gives the following description of the inner endosperm (p. 107): "Die innere, dem Embryo anliegende Endospermschicht besteht in jüngeren Samen, deren Embryonen die Mikropyle noch nicht durchbrochen haben, aus ziemlich dickwandigen, mit zahlreichen grossen Tüpfeln versehenen Zellen, welche in ihrem Aussehen etwa den Endospermzellen von *Lupinus* gleichen; . . . Später nimmt diese endospermschicht den Charakter der Schleimendosperme an." This by no means corresponds with the condition observed by the present writers. In this investigation the inner endosperm is found to be uniformly thin walled at all stages of the development of the embryo, and never at any time resembles the endosperm of *Lupinus*, and pits, if present, are quite inconspicuous.

He also suggests that the "Schleimendosperm" may be of the nature of a water reservoir. But the observations here recorded show conclusively that this endosperm is nothing other than endospermic tissue slightly altered by the action of enzymes secreted by the growing embryo, and in the process of being resorbed. Both primary and secondarily formed endosperm are used up in this fashion.

In the Buitenzorg specimens the cells of the haustorial endosperm are shown by Haberlandt (Text-figs. 1–5) as being roughly half the size of the cells of the placental (i.e., funicular, in the present terminology) tissue they invade. In the specimens here described, it was found that the cells of the haustorial endosperm were little, if any, smaller than the adjacent cells of the funicle. Another difference between the Sydney material and that examined by Haberlandt is that in the former the cells of the funicle and massive micropylar part of the integument are filled with a proteinaceous reserve food material. In unstained sections this appears as a dense yellowish mass completely filling the cells. It also absorbs dyes very readily and holds them most tenaciously. This reserve food material appears at a very early stage in the development of the ovule; it is not at any time completely exhausted by the embryo, so that even in mature ovules a proteinaceous residue still remains. Haberlandt's figures (Figs. 2–5) and description of a section through the embryo of a fruit 17 mm. or more long, show placental (i.e., funicular, in the present terminology) cells as large brown-walled cells, poor in protoplasm; the presence of proteinaceous material within them is not mentioned. In the present investigation it was found that the walls of the funicular cells remain relatively thin, so long as the associated haustoria remain functional.

2. *The nutrition of the embryo.*

In the Sydney district the time elapsing between flowering and maturation of the embryo is 9–10 months. Growth is slowest during the early developmental stages up till the time of formation of cellular endosperm. This was found to take about 7 months. As soon as the endospermic haustoria have been initiated, growth becomes relatively much more rapid. From the foregoing description of the development of the embryo, it would seem reasonable to attribute this slow initial growth to the fact that food material for the developing embryo must pass through a zone of meristematic cells. In such a case food material would be obtained with greater difficulty than if the funicle had been equipped with a vascular system, and consequently the growth would be comparatively slow. Once the endospermic haustoria are formed, however, the obtaining of food material would be much facilitated; consequently the embryo would increase rapidly in size.

3. *Comparison with* Avicennia officinalis.

As Haberlandt pointed out, there is a general resemblance between the embryos of *Avicennia* and *Aegiceras* in that, in both cases, there has been a modification of the endospermic tissue to meet the requirements of a large viviparous embryo. In *Avicennia* (Treub, 1883), a single endosperm cell, much enlarged and branched, functions as the haustorium. In *Aegiceras*, lobes of endosperm grow into the funicle tissue and function as haustoria. But there is a further resemblance between the two, which a study of the youngest stages of development of the embryo of *Aegiceras* has brought to light. In *Avicennia officinalis* the nucellus is quickly resorbed and the endosperm and embryo-sac pass gradually out through the micropyle, with the exception of a single endosperm cell which remains within the integument and grows and branches, functioning as a haustorium. In *Aegiceras majus* there is a similar tendency. The nucellus is resorbed at an early stage and the embryo-sac commences to enlarge into the micropyle. It never succeeds in growing completely out, however, since cell division in the integument enables that tissue to keep pace with the growth of the embryo-sac, until the embryo is almost mature.

4. *"Appendages."*

The function of the elongated "appendages" found in some ovaries remains obscure. They bear no resemblance to normal ovules, except in the presence of a funicle-like stalk and the size of their cells.

Summary.

1. *Aegiceras majus* Gaertn., a mangrove occurring in the Sydney district, is characterized by vivipary.

2. The carpels enclose a conical loculus in which develops the basal placenta. This placenta is expanded at the apex into an arrow-shaped head in which numerous anatropous ovules are embedded.

3. Each ovule has a massive integument. The cells in the micropylar region are potentially meristematic. The funicle is very short, and is composed of cells which are also potentially meristematic. No vascular tissue passes across the funicle to the integument.

4. The embryo-sac is elongated and is excentrically placed in the nucellus.

5. After fertilization the embryo-sac enlarges, crushing and resorbing the nucellus. It then commences to grow down into the micropyle.

6. The integument cells in this region divide rapidly radially and longitudinally. The embryo-sac continues to increase in size, absorbing the integument tissue as rapidly as it is formed.

7. Meanwhile the endosperm nucleus has divided many times so that numerous nuclei are found at the periphery of the enlarged embryo-sac.

8. The cells of the integument continue to divide, the successive divisions being so rapid that numerous folds are formed. At about this time cell formation commences at the periphery of the sac and advances inwards so that an extensive endosperm tissue is laid down. The outline of the original embryo-sac is still maintained by the cells of the integument.

9. The fertilized egg divides to form a short suspensor and embryonal tissue at the micropylar end of the sac.

10. The peripheral cells of the endosperm then become meristematic, causing general enlargement of the endosperm, until it completely fills and straightens out the integument.

11. Meanwhile the funicle has become widened by cell division, and the lower part of the endosperm grows into it in extensive folds. These folds assume a haustorial function.

12. The embryo grows rapidly, and the endosperm and integument increase in size by cell division, so that the embryo remains for a considerable period enclosed within them.

13. Finally the embryo grows through the endosperm and integument at the lower end, and the radicle and lower part of the hypocotyl emerge into the cavity of the ovary.

14. At the same time the placenta increases in length, enabling the relative position of the upper part of the embryo to remain unchanged with regard to the placental tissue, so that food continues to pass to the growing embryo.

15. The fruit is then shed from the tree. Under favourable conditions the hypocotyl continues to elongate, causing the radicle to break through the wall of the ovary and emerge.

16. Secondary roots are produced and the plant is established in the mud. The cotyledons break through the ovary wall and the plumule grows up between them.

17. Certain abnormal "appendages" which may occur on the placenta in the position of ovules are described.

18. Haberlandt's description of the endosperm of *Aegiceras majus* is discussed. A comparison is drawn between the mode of development of the embryo in *Aegiceras majus* and in *Avicennia officinalis*.

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